for a fee, bulletin board, on-line chat and newsgroup?

Programming

Further questions relating to programming include:

- Is the source code provided and is it hackable? That is, is it possible to change and modify the program?
- Does it have the facility to accept plug ins/modules/sub-routines?
- Which programming language is used?
- What standards are incorporated into the software?
- For bundled programs (in which two or more programs are packaged together in one application) how good is the integration? Is it easy to move data from one program to the other, and what interim storage is necessary when moving between programs? How are the programs integrated – what language 'glues' the components together?

Performance

The crucial test of any bioinformatic 'off-the-shelf' software is if it makes biological sense. For example, a program may be designed to find patterns, but do the patterns have any biological significance? Does the software do the job you thought it could do? Is it fit for the purpose you intended? Does it give you error-free, accurate and precise results? Can you trust the results – especially if it is a 'black box' system? What sort of quality control has been performed and does it adhere to your own company's quality control standards? Finally, is a test data set available to determine a software's performance and is it validated by a third party or from the literature?

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Nitric oxide disbalance and structural alterations of the cardiovascular system

Nearly two decades have passed since Furchgott and Zawadzki revealed the obligatory role of endothelium for arterial relaxation to acetylcholine [Furchgott, R.F. and Zawadzki, J.V. (1980) *Nature* 288, 373–376]. More than ten years have passed since endothelium-derived relaxing factor was identified as a nitric oxide (NO). At present, it is generally accepted that NO, one of the simplest molecules in nature, is actively engaged in many biological processes in the organism. The findings that NO is a potent vasodilatory molecule, continuously produced by endothelial cells, gave rise to the question of whether its decreased production might be one of the main factors contributing to the development of some types of hypertension. An experimental model of NO deficiency has been developed. The pathogenic background of NO deficiency consists of inhibition of NO synthase, an enzyme of crucial importance in the virtually ubiquitous arginine–citrulline pathway with concomitant coproduction of NO. Blockade of NO synthase disturbs the balance between vasodilatory and vasoconstrictory agents, resulting in a pronounced increase in blood pressure.

Long-term NO synthase inhibition in rats resulted not only in increased blood pressure but also in cardiac hypertrophy and an increase in arterial-wall thickness. We found that in conduit arteries – thoracic aorta, carotid artery and coronary artery – the arterial-wall thickness (tunica intima and tunica media) was increased by more than 70% [Kristek, F. and Gerová, M. (1996) *Physiol. Res.* 45, 361–367].

The question remains open as to whether these pathological changes are primary consequences of NO deficiency or whether NO deficiency only triggers the underlying processes that induce the changes. Moreover, pressure elevation itself probably also affects metabolic and proliferation processes in the vessel wall. Because NO may have an antiproliferative effect, NO synthase blockade would be expected to act in the opposite way. In NO-deficient hypertension, both stimuli – a decrease of NO level and an increase in blood pressure – seem to operate synergistically, and thus we suppose that the two factors could enhance the contribution of smooth muscle to the increase in arterial-wall mass.

From both the pathophysiological and mechanistical point of view, an important issue is whether the cellular- or the non-cellular component of the arterial wall is responsible for the described increase. Morphometric analysis of the arterial wall (tunica intima and tunica media) revealed that all of these components of the arterial wall (endothelial cells, smooth muscle cells, and extracellular matrix) increase their volume. Nevertheless, our findings indicated that the extracellular matrix significantly increased its volume and thus became the main contributor to the wall thickness [Kristek, F. *et al.* (1996) *Physiol. Res.* 45, 329–333]. Long-term NO deficiency was found to result in hypertrophic remodelling of the arterial wall. Moreover, the increased smooth-muscle contractility induced by inhibition of NO production appears to cause damage to the areas supplied by the arteries.

NO deficiency

Does supplementation of NO in the form of exogenous NO donors correct NO deficiency after NO-synthase blockade? It is well documented that exogenous donors of NO initiate a chain of events leading to vascular relaxation. However, NO donors may also be a source of superoxide radicals, and this may affect their therapeutic profile. Molsidomine, an NO donor, is frequently used to provide prophylaxis

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against anginal episodes without causing tolerance, even during long-term treatment. Thus it could be expected that long-term administration of molsidomine given together with an NO synthase inhibitor would, in comparison with NO deficiency, result not only in a significant decrease in blood pressure but also in decreased hypertrophy. Our experiments showed that blood pressure decreased significantly, yet it was still significantly higher than in controls. Cardiac hypertrophy decreased (heart/body weight was equal to the control value). Morphometric analysis of conduit arteries (thoracic aorta, carotid artery and coronary artery) revealed that the thickness of the arterial wall was the same as the controls [Kristek, F. and Gerová, M. (1998) *Pathophysiol.* 5 (Suppl. 1), 47]. The results indicated that despite the complex action of exogenous donors, NO supplementation can correct, at least in part, endogenous NO deficiency. This therefore supports the increasing body of knowledge supporting the pharmacotherapeutic use of NO donors in the treatment of cardiovascular diseases.

Various experimental models of hypertension, including spontaneously hypertensive rats (SHR), are also characterized by cardiac hypertrophy and hypertrophy of the arterial wall. We addressed the question of whether availability, and possibly transport and metabolism, of L-arginine (substrate for NO synthase) may limit NO production, resulting, at least partially, in the mentioned pathological alterations. Spontaneously hypertensive rats received L-arginine together with NO synthase inhibitor over a long period. We found no attenuation in either blood pressure or cardiac- and arterial-wall hypertrophy in SHR [Kristek, F. (1998) *Exp. Physiol.* 83, 595–603]. Despite the findings that L-arginine lowered blood pressure and increased indicators of NO production (plasma citrulline, urinary excretion of nitrite and nitrate) in some types of hypertension, it seems that pathological changes in this type of hypertension (SHR) are not based on a shortage of the substrate (L-arginine) for NO production. To resolve this problem, it would be helpful to know the actual concentrations of NO *in vivo*. Unfortunately, a reliable and simple method for NO measurement is still not available. Nevertheless, it is necessary to bear in mind that L-arginine and NO synthase are not necessarily the only limiting factor in NO production. Other factors, such as nonenzymatic generation of NO, may also play an important role.

Summary

Nitric oxide is an important vasoactive molecule that enters actively into the control processes of the cardiovascular system. Misbalance in its production results in pathological changes. This problem can be traced back over a considerable period of time, as more than 100 years ago organic nitrates were introduced into clinical practice. There is, however, much work to be done in order to complete our understanding of the role of NO in the cardiovascular system.

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